PATENT SPECIFICATION

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COMPLETE SPECIFICATION

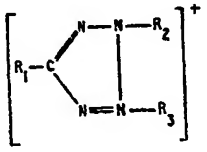
Improvements in or relating to Tetrazolium Compounds

We, MAY & BAKER LIMITED, a British Company of Dagenham, Essex, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is for improvements in or relating to tetrazolium salts and to processes for the preparation of these compounds.

It is the object of this invention to provide new tetrazolium salts which possess therapeutic activity.

The tetrazolium salts of the present invention contain the cation represented by general formula I:



I

wherein R_1 represents an alkyl, aryl or substituted aryl radical, R_2 and R_3 are the same or different and each represents an aryl or substituted aryl radical, at least one of the aromatic nuclei of R_2 and R_3 being substituted in the *para*-position by a group represented by the formula $-A-R_4$, in which $-A-$ represents $-CH=CH-$ or $-N=N-$, and R_4 represents an aryl or substituted aryl radical.

Where substituted, the various aryl radicals, which are preferably all phenyl nuclei, may carry one or more halogen, nitro, hydroxy, alkyl, acylamino, dialkylamino, quaternary ammonium, carboxy or alkoxy substituent groups.

According to a feature of the present invention, the new tetrazolium salts are produced by the oxidation of the formazans conforming to the conventional formula II:

$$R_2-NH-N=C(R_1)-N=N-R_3 \quad \text{II}$$

for example 1:3-diphenyl-5-(*p*-styrylphenyl)-formazan, with a dehydrogenating agent such as lead tetra-acetate, mercuric oxide or *iso*-amyl nitrite and conversion of the product to the appropriate salt. Preferably, the formazan is treated with the dehydrogenating agent in the presence of a solvent for the formazan, and the reaction mixture is then acidified to convert the tetrazolium compound formed into an appropriate salt.

The aforesaid formazan starting materials may be prepared in accordance with the process described in the specification of copending Application No. 7399/54 (Serial No. 738,632) viz. by diazotising an amine of the formula R_3NH_2 to form a compound of the formula:

$$R_3-N=N-X \quad \text{III}$$

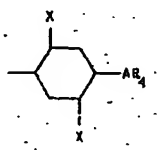
where X is an anion of an acid used in diazotisation reactions such as chloride or sulphate, and coupling the product in a basic medium (preferably in pyridine and preferably at a temperature below 15° C.) with a compound of the general formula:

$$R_2-NH-N=CHR_1 \quad \text{IV}$$

where R_1, R_2 and R_3 have the significance given above.

The new compounds of the present invention have been found to possess useful therapeutic activity in the treatment of infections caused by tubercle bacilli, pathogenic fungi or viruses, in particular those tetrazolium compounds in which R_1 represents a lower alkyl or phenyl group, or a phenyl group substituted in the *para*-position by a halogen

atom or by a hydroxy, lower alkoxy or di-(lower alkyl)-amino group, R_2 represents a phenyl or *p*-acetamido-phenyl group and R_3 represents the group:



where A has the significance hereinbefore defined, X represents a hydrogen atom or a lower alkyl group and R_4 represents a phenyl group or a phenyl group substituted in the *para*-position by a halogen atom or by a lower alkyl, hydroxy or nitro group.

New compounds of the invention possessing anti-tubercular activity include the following:

2:5 - diphenyl - 3 - (2¹:5¹ - dimethyl-4¹-phenylazo-phenyl)-tetrazolium salts, e.g. the chloride,

2:5-diphenyl - 3 - [4¹-(4¹¹-hydroxyphenylazo)-phenyl]- tetrazolium salts, e.g. the isethionate,

2:5-diphenyl - 3 - [4¹ - (4¹¹-methylphenylazo)-phenyl] - tetrazolium salts, e.g. the isethionate,

2-phenyl - 5 - (4¹ - dimethyl(aminophenyl)-3 - [4¹ - (4¹¹ - hydroxyphenylazo) - phenyl]-tetrazolium salts, e.g. the chloride methochloride,

2-(4¹ - acetamidophenyl) - 5 - phenyl-3-(4¹-phenylazophenyl)-tetrazolium salts, e.g. the isethionate, and

2:5 - diphenyl- 3 - (4¹-styrylphenyl) -tetrazolium salts, e.g. the chloride.

New compounds of the invention active in high dilutions against pathogenic fungi such as *Actinomyces maduræ* include the following, of which the first four named compounds are particularly active:

2-phenyl - 5 - (4¹ - methoxyphenyl)-3-(4¹-styrylphenyl)-tetrazolium salts, e.g. the iodide,

2-phenyl - 5 - (4¹ - bromophenyl) - 3 - (4¹-styrylphenyl)-tetrazolium salts, e.g. the iodide,

2:5-diphenyl - 3 - (4¹ - styrylphenyl)-tetrazolium salts, e.g. the chloride,

2:5-diphenyl - 3 - [4¹ - (4¹¹-chlorophenylazo) - phenyl] - tetrazolium salts, e.g. the chloride,

2:5-diphenyl - 3 - [4¹-(4¹¹-nitrophenylazo)-phenyl]-tetrazolium salts, e.g. the chloride, and

2-phenyl - 5 - methyl- 3 -(4¹-styrylphenyl)-tetrazolium salts, e.g. the iodide.

The present invention is illustrated by the following non-limitative Examples:—

EXAMPLE I.

A solution of 1:3-diphenyl-5-(4¹-phenylazophenyl)-formazan (9.6 g.) in dry chloroform (50 c.c.) was boiled for 2 hours with lead tetra-acetate (12 g.) All the solvent was then evaporated, the residual dark brown oil was dissolved in boiling water and the solution was filtered with the aid of a little Hyflo Supercel (the words "Hyflo Supercel" are registered Trade Marks). Concentrated hydrochloric acid was then added drop by drop to the filtrate until no further precipitation occurred. The precipitate, which was a mixture of lead chloride and 2:5-diphenyl-3-(4¹-phenylazophenyl) - tetrazolium chloride, was filtered off and extracted with methanol. Addition of ether to the methanolic filtrate yielded the tetrazolium chloride as an oil, which soon solidified to an orange solid. This was collected, dissolved in hot water, and converted to the iodide by adding an aqueous solution of potassium iodide. The iodide crystallised from methanol in orange rods, m.p. 231—232° C. (decomp.). This iodide may be converted into other salts, e.g. chloride, by standard methods.

Similarly prepared were:—

1) 2:5-Diphenyl- 3 -[4¹-(4¹¹-nitrophenylazo)-phenyl]-tetrazolium chloride dihydrate from 1:3-diphenyl - 5 - [4¹-(4¹¹-nitrophenylazo)-phenyl] - formazan, m.p. 210° C. (decomp.). This compound, which forms orange-red prisms, decomposes at different temperatures according to the rate of heating.

2) 2-(4¹-Chlorophenyl) - 5 - phenyl-3-(4¹-phenylazophenyl) - tetrazolium iodide, m.p. 218° C. (decomp.) from 1-(4¹-chlorophenyl)-3-phenyl - 5 - (4¹-phenylazophenyl)-formazan, m.p. 168—170° C.

3) 2-(4¹ - Acetamidophenyl) - 5 - phenyl-3-(4¹ - phenylazophenyl) - tetrazolium iodide, m.p. 258° C. (decomp.) from 1-(4¹-acetamidophenyl)-3-phenyl - 5 - (4¹-phenylazophenyl)-formazan, m.p. 215° C. This was converted to the isethionate, an orange solid, by treatment with silver isethionate in boiling methanol.

EXAMPLE II.

1:3 - Diphenyl - 5 - (4¹ - phenylazo -1¹-naphthyl)-formazan (4.0 g.) in chloroform (100 c.c.) was heated with lead tetra-acetate (4.5 g.) under reflux for 30 minutes and the solvent then evaporated. The residue was treated with excess dilute hydrochloric acid and amyl alcohol. The organic layer was separated, washed with water, dried, and removal of the solvent left crude 2:5-diphenyl-3-(4¹-phenylazo-1¹-naphthyl)-tetrazolium chloride. Dissolving in hot water followed by addition of aqueous potassium iodide gave the very sparingly soluble iodide m.p. 180° C. (decomp.), which crystallised from aqueous alcohol in small orange needles. The iodide can be reconverted to the corresponding chloride by known methods.

Similarly prepared, from 1 - phenyl -3-methyl - 5 - (4¹ - phenylazophenyl)-formazan, was 2 - phenyl - 5 - methyl-3-(4¹-phenylazo-

phenyl) - tetrazolium iodide. m.p. 127—129° C.

EXAMPLE III.

1:3 - Diphenyl - 5 - [4¹-(4¹¹-chlorophenylazo)-phenyl]-formazan (5.0 g.) in methanol (50 c.c.) was mixed with yellow mercuric oxide (15 g.) and refluxed for about 30 minutes. The resulting solution was filtered and sufficient dilute hydrochloric acid added to make it weakly acid to litmus, followed by water (25 c.c.). Filtration through a little "Hyflo Supercel" followed by evaporation to dryness gave a red gum which slowly crystallised. The crude 2:5-diphenyl-3-[4¹-(4¹¹ - chlorophenylazo)-phenyl] - tetrazolium chloride was dissolved in ethanol and crystallised by the addition of ether as red prisms or needles, m.p. 184—185° C. (decomp.).

Similarly prepared were:—

1) 2:5-Diphenyl-3-[4¹-(4¹¹-methylphenylazo)-phenyl]-tetrazolium iodide, m.p. 175—177° C. (decomp.) from 1:3 - diphenyl-5-[4¹-(4¹¹-methylphenylazo) - phenyl]-formazan, m.p. 186—188° C., adding excess of 10% potassium iodide solution after the addition of hydrochloric acid to precipitate the tetrazolium iodide. The isethionate was prepared by refluxing the iodide (2.84 g.) in dry ethanol (50 c.c.) with powdered silver isethionate (1.21 g.) for 6 hours, filtering and evaporating to dryness. The isethionate remained as a red glittering powder of glassy appearance whose melting point could not be taken.

2) 2 - (4¹-Carboxyphenyl) - 5 - phenyl-3-(4¹-phenylazophenyl) - tetrazolium chloride, m.p. 164° C. (decomp.), from 1-(4¹carboxyphenyl)-3-phenyl - 5 - (4¹ - phenylazophenyl)-formazon, m.p. 209° C.

3) 2:5 - Diphenyl-3-(2¹:5¹-dimethyl-4¹-phenylazophenyl) - tetrazolium chloride trihydrate, m.p. 65° C., from 1:3-diphenyl-5-(2¹:5¹-dimethyl-4¹-phenylazophenyl) - formazan, m.p. 197° C.

EXAMPLE IV.

2:3-Diphenyl - 5 - [4¹-(4¹¹-hydroxyphenylazo)-phenyl]-formazan (10 g.) was suspended in ethanol (200 c.c.) and *iso*-amyl nitrite (10 c.c.) added. The suspension was treated with hydrogen chloride at 0° C. for 30 minutes, when the formazan dissolved to give a light reddish brown solution. This was poured into a litre of water, stirred, the water decanted and the dark tarry residue taken into methanol charcoaled, filtered and precipitated carefully with ether. The 2:5-diphenyl-3-[4¹ - (4¹¹ - hydroxyphenylazo) - phenyl]-tetrazolium chloride crystallised in orange red conglomerates, m.p. 230° C. (decomp.). This product (1.75 g.) was treated with silver isethionate (0.9 g.) in dry ethanol (25 c.c.) to yield the corresponding isethionate, deep ruby red prisms crystallising slowly from water, m.p. 218—219° C. (decomp.).

Similarly prepared were:—

1) 2:5 - Diphenyl- 3 -[4¹-(2¹¹-chloro-4¹¹-hydroxyphenylazo) - phenyl] - tetrazolium chloride, m.p. 204—205° C. (decomp.) from 1:3-diphenyl -5- [4¹-(2¹¹-chloro-4¹¹-hydroxyphenylazo)-phenyl] - formazan, m.p. 149—150° C.

2) 2:5-Diphenyl -3- [4¹ - (3¹¹-chloro-4¹¹-hydroxyphenylazo) - phenyl] - tetrazolium chloride, m.p. 206—207° C. (decomp.), from 1:3-diphenyl -5- [4¹-(3¹¹-chloro-4¹¹-hydroxyphenylazo)-phenyl] - formazan, m.p. 205—210° C.

3) 2-Phenyl -5- (4¹ - hydroxyphenyl) -3-(4¹-phenylazophenyl) - tetrazolium chloride, m.p. 267° C., from 1-phenyl-5-(4¹-phenylazophenyl) -3- (4¹-hydroxyphenyl) - formazan, m.p. 181° C.

EXAMPLE V.

1 - Phenyl -3- (4¹ - dimethylaminophenyl)-5 - [4¹ - (4¹ᴱ - hydroxyphenylazo) - phenyl]-formazan methochloride (3.6 g.) was oxidised in methanol with *iso*-amyl nitrite (4 c.c.) in the usual way and, after removal of solvents, digested with hot *iso*-propanol. The 2-phenyl-5 - (4¹ - dimethylaminophenyl) - 3 - [4¹-(4¹¹-hydroxyphenylazo) - phenyl] - tetrazolium chloride methochloride was then crystallised from methanol/ether as an orange crystalline powder, m.p. 211—212° C. (decomp.).

EXAMPLE VII.

A mixture of 1:3-diphenyl-5-(4¹-styrylphenyl)-formazan (1.0 g.) in methyl alcohol (50 c.c.) containing yellow mercuric oxide (4.0 g.) was heated under reflux until the formazan colour disappeared. The yellow solution obtained after filtration was diluted with water, again filtered, acidified with concentrated hydrochloric acid and the methanol removed by distillation. The residual oil rapidly solidified and the product, 2:5-diphenyl - 3 - (4¹-styrylphenyl) - tetrazolium chloride, m.p. 228—229° C. (decomp.), recrystallised from water.

The chloride was converted into the corresponding isethionate (m.p. 181° C.) by passing it in aqueous solution over an ion exchange medium (Amberlite) to provide a solution of the corresponding hydroxide which was then treated with isethionic acid. The corresponding sulphate (dihydrate, decomposing on heating) and bisulphate (m.p. 169—171° C.) were each prepared by treating the chloride with the silver salt of the corresponding acid.

Similarly prepared, but in most cases precipitated as the iodides, were:—

1) 2:5 - Diphenyl -3- [4¹ - (4¹¹ - acetylaminostyryl) - phenyl] - tetrazolium iodide monohydrate, m.p. 244° C. (decomp.), from 1:3-diphenyl - 5 - [4¹-(4¹¹-acetylaminostyryl)-phenyl]-formazan, m.p. 208—209° C.

2) 2:5 - Diphenyl - 3 - [4¹ - (4¹¹-bromostyryl)-phenyl] - tetrazolium chloride monohydrate, m.p. 216—217° C. (decomp.), from 1:3 - diphenyl - 5 - [4¹ - (4¹¹ - bromostyryl)-phenyl]-formazan, m.p. 186—187° C.

3) 2:5 - Diphenyl - 3 - [4¹-(4¹¹-hydroxystyryl)-phenyl]-tetrazolium iodide, m.p. 272° C. (decomp.) from 1:3-diphenyl-5-[4¹-(4¹¹-hydroxystyryl)-phenyl]-formazan, m.p. 175—176° C.

4) 2 - Phenyl - 5 - (4¹ - bromophenyl)-3-(4¹ -styrylphenyl)-tetrazolium iodide, m.p. 206° C. (decomp.), from 1 - phenyl -3- (4¹-bromophenyl) - 5 - 4¹-styrylphenyl)-formazan m.p. 170—171° C. (decomp.).

5) 2-Phenyl- 5 -(4¹-methoxyphenyl)-3-(4¹-styrylphenyl)-tetrazolium iodide, m.p. 167—168° C. (decomp.), from 1-phenyl- 3 -(4¹-methoxyphenyl)- 5 -(4¹-styrylphenyl) - formazan, m.p. 157—158° C.

6) 2:5-Diphenyl- 3 -[4¹-(4¹¹-nitrostyryl)-phenyl] - tetrazolium chloride dihydrate, m.p. 233—234° C. (decomp.), from 1:3-diphenyl-5-[4¹-(4¹¹-nitrostyryl)-phenyl]-formazan, m.p. 185—186° C.

7) 2-Phenyl- 5 -methyl-3-(4¹-styrylphenyl)-tetrazolium iodide, hemihydrate, m.p. 169—171° C., from 1-phenyl-3-methyl-5-(4¹-styrylphenyl) - formazan, m.p. 160—162° C. (decomp.).

8) 2-Phenyl- 5 -methyl- 3 -[4¹-(4¹¹-nitrostyryl)-phenyl]-tetrazolium iodide, m.p. 222—223° C. (decomp.), from 1-phenyl-3-methyl-5 - [4¹ - (4¹¹-nitrostyryl)-phenyl] - formazan, m.p. 182—183° C.

EXAMPLE VII

1-(4¹-Phenylazophenyl)- 3 -phenyl- 5 -(4¹-styrylphenyl)-formazan (4 g.) was refluxed in methanol (60 c.c.) with yellow mercuric oxide (15 g.) after adding a little methanol, until the colour was discharged. After filtration, the solution was poured into dilute hydriodic acid, giving a red tar which was crystallised from ethanol after charcoaling, yielding 2-(4¹-phenylazophenyl) - 5 - phenyl - 3 - (4¹-styrylphenyl)-tetrazolium iodide, m.p. 172—176° C. (decomp.).

EXAMPLE VIII

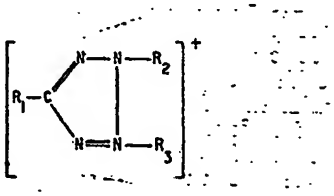
1-(4¹-Phenylazophenyl) - 3 - (4¹ - acetoxyphenyl)-5-(4¹-styrylphenyl)-formazan (2.0 g.) was reacted in ethanol (40 c.c.) with *iso*-amyl nitrite (4 c.c.) and hydrogen chloride gas until decolourised. The solution was poured into water, filtered and warmed with dilute hydrochloric acid. Thee residual gum was eluted with hot acetone and precipitated with ether. 2-(4¹-Phenylazophenyl) - 3 - (4¹-styrylphenyl)-5 - (4¹-hydroxyphenyl) - tetrazolium chloride was crystallised from acetone/ether containing a few drops of methanol m.p. 215—216° C.

EXAMPLE IX

1-(4¹-Phenylazophenyl) - 3 - (4¹ - carboxyphenyl)-5-(4¹-styrylphenyl)-formazan (18.5 g.) was oxidised in the usual way with *iso*-amyl nitrite and hydrogen chloride to yield 2-(4¹-phenylazophenyl) - 3 - (4¹-styrylphenyl)-5-(4¹-carboxyphenyl)-tetrazolium chloride as a red microcrystalline solid, recrystallised from ethanol, m.p. 195—196° C. (decomp.).

What we claim is:—

1. Tetrazolium salts, the cations of which conform to the formula:



wherein R_1 represents an alkyl, aryl or substituted aryl radical, R_2 and R_3 are the same or different and each represents an aryl or substituted aryl radical, at least one of the aromatic nuclei of R_2 and R_3 being substituted in the *para*-position by a group represented by the formula $-A-R_4$, in which $-A-$ represents $-CH=CH-$ or $-N=N-$, and R_4 represents an aryl or substituted aryl radical.

2. Tetrazolium salts as claimed in claim 1 wherein the various aryl radicals are all phenyl radicals and at least one of the phenyl radicals contains one or more substituents selected from halogen, nitro, hydroxy, alkyl, acylamino, dialkylamino, quaternary ammonium, carboxy and alkoxy.

3. Chemical compounds as claimed in claim 2 wherein each phenyl radical contains one of the specified substituents in the *para*-position.

4. 2:5-Diphenyl - 3 - (2¹:5¹-dimethyl-4¹-phenylazophenyl)-tetrazolium salts.

5. 2:5-Diphenyl - 3 - [4¹ - (4¹¹-hydroxyphenylazo)-phenyl]-tetrazolium salts.

6. 2:5-Diphenyl - 3 - [4¹ - (4¹¹ - methylphenylazo)-phenyl]-tetrazolium salts.

7. 2-Phenyl- 5 -(4¹-dimethylaminophenyl)-3 - [4¹-(4¹¹-hydroxyphenylazo)-phenyl] - tetrazolium salts.

8. 2- (4¹-Acetamidophenyl) - 5 - phenyl-3-(4¹-phenylazophenyl)-tetrazolium salts.

9. 2:5 - Diphenyl - 3 - (4¹-styrylphenyl)-tetrazolium salts.

10. 2-Phenyl - 5 - (4¹-methoxyphenyl)-3-(4¹-styrylphenyl)-tetrazolium salts.

11. 2-Phenyl - 5 - (4¹ - bromophenyl) -3-(4¹-styrylphenyl)-tetrazolium salts.

12. 2:5-Diphenyl - 3 - [4¹ - (4¹¹-chlorophenylazo)-phenyl]-tetrazolium salts.

13. 2:5-Diphenyl- 3 -[4¹-(4¹¹-nitrophenylazo)-phenyl]-tetrazolium salts.

14. 2-Phenyl - 5 - methyl - 3 - (4¹ - styrylphenyl)-tetrazolium salts.

15. A process for preparing the tetrazolium salts claimed in claim 1 which comprises coupling a compound having the general formula:

$$R_3-N=N-X$$

where X is an anion of an acid used in diazotitisation reactions such as chloride or sulphate, with a compound having the general formula:

$R_2-NH-N=CHR_1$

(R_1, R_2 and R_3 having the significance set forth in claim 1), and oxidising the resultant formazan with a dehydrogenating agent.

16. A process as claimed in claim 15 in which the formazan is heated with the dehydrogenating agent in the presence of a solvent for the formazan, and the reaction mixture is then acidified to convert the tetrazolium compound formed into an appropriate salt.

17. A process as claimed in claim 15 or 16 wherein the dehydrogenating agent is lead tetra-acetate, mercuric oxide or *iso*-amyl nitrite.

18. Processes for the preparation of tetrazolium salts as claimed in claim 1 substantially as described in the foregoing Examples.

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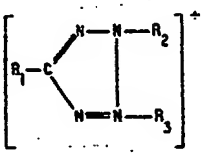
PROVISIONAL SPECIFICATION

Improvements in or relating to Tetrazolium Compounds

We, MAY & BAKER LIMITED, a British Company of Dagenham, Essex, do hereby declare this invention to be described in the following statement:—

This invention is for improvements in or relating to tetrazolium salts and to processes for their preparation and has for its object to provide new and therapeutically useful substances.

The new compounds of the present invention are tetrazolium salts which contain the cation represented by the general formula:



in which R_1 represents an alkyl or aryl radical, R_2 and R_3 each represents an aryl radical and in which at least one of the aromatic nuclei is substituted in the *para*-position by a group represented by the formula $-A-R_4$ in which $-A-$ represents $-CH=CH-$ or $-N=N-$, and R_4 represents an aryl radical. The aromatic nuclei may in addition carry one or more substituents, for example, halogen, nitro, hydroxy, alkyl, amino, quaternary ammonium, carboxy or alkoxy groups. The aryl radicals are preferably all phenyl radicals.

The new compounds of the present invention have been shown to possess useful therapeutic activity, in particular those represented by the general formula when R_1 and R_2 are phenyl groups and R_3 is a *p*-phenylazophenyl group, such as for example 2:5-diphenyl-3-[2^1:5^1 - dimethyl - 4^1 - phenylazophenyl]-tetrazolium chloride, 2-phenyl - 3 - [4^1-(4^{11}-hydroxyphenylazo) - phenyl]-5-(4^1-dimethylaminophenyl) - tetrazolium chloride methochloride, and 2:5 - diphenyl - 3 - [4^1 - (4^{11}-hydroxyphenylazo)-phenyl]-tetrazolium isethionate, possess anti-tubercular and virucidal activity while those represented by the general formula when R_1 and R_2 are phenyl groups and R_3 is a *p*-styryl-phenyl group, such as 2:5-diphenyl-3-(4^1-styryl-phenyl)-tetrazolium chloride, are also active in high dilutions against pathogenic fungi such as *Actinomyces maduræ*.

According to a feature of the present invention, the new compounds may be produced by the oxidation of the corresponding formazans of the general formulæ:

$R_2-NH-N=C(R_1)-N=N-R_3$ or
$R_2-N=N-C(R_1)=N-NH-R_3$,

for example 1:3-diphenyl-5-(*p*-styrylphenyl)-formazan, with a dehydrogenating agent such as lead tetra-acetate, mercuric oxide or *iso*-amyl nitrite and conversion of the product to the appropriate salt.

According to a preferred feature of the above process, the formazan is heated with the dehydrogenating agent in the presence of a solvent for the formazan, and the reaction mixture is then acidified to convert the tetrazolium compound formed into an appropriate salt.

The present invention is illustrated by the following non-limitative Examples:—

EXAMPLE I.

A solution of 1:3-diphenyl-5-(4^1-phenylazophenyl)-formazan (9.6 g.) in dry chloroform (50 c.c.) was boiled for 2 hours with lead tetra-acetate (12 g.). All the solvent was then evaporated, the residual dark brown oil was dissolved in boiling water and the solution was filtered with the aid of a little Hyflo Supercel. Concentrated hydrochloric acid was then added drop by drop to the filtrate until no further precipitation occurred. The precipitate, which was a mixture of lead chloride and 2:5-diphenyl - 3 - (4^1-phenylazophenyl)-tetrazolium chloride, was filtered off and extracted with methanol. Addition of ether to the methanolic filtrate yielded the tetrazolium chloride as an oil, which soon solidified to an orange solid. This was collected, dissolved in hot water, and converted to the iodide by adding an aqueous solution of potas-

sium iodide. The iodide crystallised from methanol in orange rods, m.p. 231—232° C. (decomp.). This iodide may be converted into other salts, e.g. chloride, by standard methods.

The intermediate formazan was prepared in the following way:—A solution of *p*-aminoazobenzene hydrochloride (17.25 g.) in concentrated hydrochloric acid (11.2 c.c.) and water (25 c.c.) was cooled to 0—5° C. and diazotised with sodium nitrite (5.25 g.) in water (10 c.c.). Ethanol (20 c.c.) was then added, and the resulting mixture added gradually to a stirred solution of benzaldehyde phenylhydrazone (9.8 g.) in pyridine (100 c.c.). After stirring for a further 3 hours and then warming to room temperature, the 1:3-diphenyl - 5 - (4¹-phenylazophenyl)-formazan was filtered off, washed with dilute hydrochloric acid, water, and ethanol, then boiled with ethanol (100 c.c.), cooled, and finally filtered. The resulting solid was used directly in the next stage, but if desired it may be recrystallised from hot acetone by careful addition of water as purplish black needles, m.p. 182° C. (decomp.).

Similarly prepared were:—

1) 2:5-Diphenyl-3-[4¹ - (4¹¹-nitrophenylazo)-phenyl]-tetrazolium chloride dihydrate from 1:3-diphenyl - 5 - [4¹-(4¹¹-nitrophenylazo)-phenyl] - formazan, m.p. 210° C. (decomp.). This compound, which forms orange-red prisms, decomposes at different temperatures according to the rate of heating.

2) 2-(4¹-Chlorophenyl) - 5 - phenyl-3-(4¹-phenylazophenyl) - tetrazolium iodide, m.p. 218° C. (decomp.) from 1-(4¹-chlorophenyl)-3-phenyl - 5 - (4¹-phenylazophenyl)-formazan, m.p. 168—170° C.

3) 2-(4¹ - Acetamidophenyl) - 5 - phenyl-3-(4¹ - phenylazophenyl) - tetrazolium iodide, m.p. 258° (decomp.) from 1-(4¹-acetamidophenyl)-3-phenyl - 5 - (4¹-phenylazophenyl)-formazan, m.p. 215°. This was converted to the isethionate, an orange solid, by treatment with silver isethionate in boiling methanol.

EXAMPLE II.

1:3 - Diphenyl - 5 - (4¹ - phenylazo - 1¹-naphthyl-formazan (4.0 g.) in chloroform (100 c.c.) was heated with lead tetra-acetate (4.5 g.) under reflux for 30 minutes and the solvent then evaporated. The residue was treated with excess dilute hydrochloric acid and amyl alcohol. The organic layer was separated, washed with water, dried, and removal of the solvent left crude 2:5-diphenyl-3-(4¹-phenylazo-1¹-naphthyl)-tetrazolium chloride. Dissolving in hot water followed by addition of aqueous potassium iodide gave the very sparingly soluble iodide m.p. 180° C. (decomp.), which crystallised from aqueous alcohol in small orange needles. The iodide can be reconverted to the corresponding chloride by known methods.

The intermediate formazan was prepared in the following way:—4 - Phenylazonaphthylamine-1 (26.7 g.) was diazotised with sodium nitrite in a mixture of glacial acetic and sulphuric acids. The diazonium solution was added slowly with stirring to benzaldehyde phenylhydrazone (19.6 g.) in pyridine (600 c.c.), at a temperature of less than 10° C. After some time the solution was diluted with water and the resultant dark solid was filtered off, washed and dried. Recrystallisation from chloroform gave the pure formazan as purple needles, m.p. 190—192° C.

EXAMPLE III.

Starting from acetaldehyde phenylhydrazone and diazotised *p* - aminoazobenzene, 1-phenyl-3-methyl- 5 -(4¹ - phenylazophenyl)-formazan, reddish brown crystals m.p. 110° C., was prepared and oxidised with lead tetra acetate to the tetrazolium salt, isolated as 2-phenyl- 5 -methyl - 3 - (4¹-phenylazophenyl)-tetrazolium iodide from alcohol/ether, m.p. 127—129° C.

EXAMPLE IV.

1:3 - Diphenyl - 5 - [4¹-(4¹¹-chlorophenylazo)-phenyl]-formazan (5.0 g.) in methanol (50 c.c.) was mixed with yellow mercuric oxide (15 g.) and refluxed for about 30 minutes. The resulting solution was filtered and sufficient dilute hydrochloric acid added to make it weakly acid to litmus, followed by water (25 c.c.). Filtration through a little Hyflo Supercel followed by evaporation to dryness gave a red gum which slowly crystallised. The crude 2:5-diphenyl-3-[4¹-(4¹¹ - chlorophenylazo)-phenyl] - tetrazolium chloride was dissolved in ethanol and crystallised by the addition of ether as red prisms or needles, m.p. 184—185° C. (decomp.).

The intermediate formazan was prepared in the following way:—4¹-Chloro-4-amino-azobenzene (11.0 g.) was dissolved in glacial acetic acid (100 c.c.) and concentrated sulphuric acid (5.4 c.c.) added. A solution of dried sodium nitrite (3.5 g.) in concentrated sulphuric acid (10 c.c.) and glacial acetic acid (50 c.c.) was added at 0° C. with stirring. The diazonium solution was added slowly with stirring to a solution of benzaldehyde phenylhydrazone (9.5 g.) in pyridine (400 c.c.) cooled below 10° C. After standing overnight, the solution was treated with water and the almost black mass of felted needles was filtered, washed with water and methanol and dried. Crystallisation from ethyl acetate yielded a black solid with green lustre, m.p. 194.5—195° C.

Similarly prepared were:—

1) 2:5-Diphenyl-3-[4¹-(4¹¹-methylphenylazo)-phenyl]-tetrazolium iodide, m.p. 175—177° C. (decomp.) from 1:3 - diphenyl-5-[4¹-(4¹¹-methylphenylazo) - phenyl]-formazan, m.p. 186—188° C., adding excess of 10% potassium iodide solution after the addition of hydrochloric acid to precipitate the tetra-

zolium iodide. The isethionate was prepared by refluxing the iodide (2.84 g.) in dry ethanol (50 c.c.) with powdered silver isethionate (1.21 g.) for 6 hours, filtering and evaporating to dryness. The isethionate remained as a red glittering powder of glassy appearance whose melting point could not be taken.

2) 2 - (4¹-Carboxyphenyl) - 5 - phenyl-3-(4¹-phenylazophenyl) - tetrazolium chloride, m.p. 164° C. (decomp.), from 1-(4¹-carboxyphenyl)-3-phenyl - 5 - (4¹ - phenylazophenyl)-formazan, m.p. 209° C.

3) 2:5 - Diphenyl -3-(2¹:5¹-dimethyl-4¹-phenylazophenyl) - tetrazolium chloride trihydrate, m.p. 65° C., from 1:3-diphenyl-5-(2¹:5¹-dimethyl-4¹-phenylazophenyl) - formazan, m.p. 197° C.

EXAMPLE V.

2:3-Diphenyl - 5 -[4¹-(4¹¹-hydroxyphenylazo)-phenyl]-formazan (10 g.) was suspended in ethanol (200 c.c.) and *iso*-amyl nitrite (10 c.c.) added. The suspension was treated with hydrogen chloride at 0° C. for 30 minutes, when the formazan dissolved to give a light reddish brown solution. This was poured into a litre of water, stirred, the water decanted and the dark tarry residue taken into methanol, charcoaled, filtered and precipitated carefully with ether. The 2:5-diphenyl-3-[4¹ - (4¹¹ - hydroxyphenylazo) - phenyl]-tetrazolium chloride crystallised in orange red conglomerates, m.p. 230° C. (decomp.). This product (1.75 g.) was treated with silver isethionate (0.9 g.) in dry ethanol (25 c.c.) to yield the corresponding isethionate, deep ruby red prisms crystallising slowly from water, m.p. 218—219° C. (decomp.).

The intermediate formazan was prepared as follows:—4¹ - hydroxy - 4 - aminoazobenzene (13.1 g.) was dissolved in a mixture of glacial acetic acid (100 c.c.) and concentrated sulphuric acid (6 c.c.) and diazotised at 0° C. with a solution of sodium nitrite (4.3 g.) in concentrated sulphuric acid (72 c.c.). The diazonium solution was added slowly with stirring to a solution of benzaldehyde phenylhydrazone (12.2 g.) in pyridine (400 c.c.) so that the temperature did not exceed 15° C. After standing overnight, an equal volume of water was added and the formazan thereby precipitated. It was washed with water and then methanol and crystallised from ethanol to give dark purple fine needles with a reddish lustre, m.p. 193—194° C.

Similarly prepared were:—

1) 2:5 - Diphenyl- 3 -[4¹-(2¹¹-chloro-4¹¹-chloride, m.p. 204—205° C. (decomp.) from 1:3-diphenyl -5- [4¹-(2¹¹-chloro-4¹¹-hydroxyphenylazo)-phenyl] - formazan, m.p. 149—150° C.

2) 2:5-Diphenyl -3- [4¹ - (3¹¹-chloro-4¹¹-hydroxyphenylazo) - phenyl] - tetrazolium chloride, m.p. 206—207° C. (decomp.), from 1:3-diphenyl -5- [4¹-(3¹¹-chloro-4¹¹-hydroxyphenylazo) - phenyl]-formazan, m.p. 205—210° C.

3) 2-Phenyl -5- (4¹ - hydroxyphenyl) -3-(4¹-phenylazophenyl) - tetrazolium chloride, m.p. 267° C., from 1-phenyl-5-(4¹-phenylazophenyl) -3- (4¹-hydroxyphenyl) - formazan, m.p. 181° C. (The formazan was prepared from diazotised *p*-aminoazobenzene and *p*-acetoxybenzaldehyde phenyl hydrazone and the product, m.p. 191° C., was hydrolysed with $\frac{N}{5}$ — aqueous-ethanolic sodium hydroxide).

EXAMPLE VI.

1 - Phenyl -3- (4¹ - dimethylaminophenyl)-5 - [4¹ - (4¹¹ - hydroxyphenylazo) - phenyl]-formazan methochloride (3.6 g.) was oxidised in methanol with *iso*-amyl nitrite (4 c.c.) in the usual way and, after removal of solvents, digested with hot *iso*-propanol. The 2-phenyl-3 - (4¹ - dimethylaminophenyl) - 5 - [4¹-(4¹¹-hydroxyphenylazo) - phenyl] - tetrazolium chloride methochloride was then crystallised from methanol/ether as an orange crystalline powder, m.p. 211—212° C. (decomp.).

The intermediate formazan was prepared as follows: 4 - Hydroxy-4¹-aminoazobenzene (53 g.) was diazotised in concentrated hydrochloric acid (255 c.c.) with sodium nitrite (17.5 g.) and coupled under the usual conditions with *p* - dimethylaminobenzaldehyde phenylhydrazone methiodide (90 g.) dissolved in pyridine (500 c.c.). The dark purplish red crystals, m.p. 172—173° C. (decomp.) were mainly the methochloride, contaminated with a small amount of the methiodide.

EXAMPLE VII

A mixture of 1:3-diphenyl-5-(4¹-styrylphenyl)-formazan (1.0 g.) in methyl alcohol (50 c.c.) containing yellow mercuric oxide (4.0 g.) was heated under reflux until the formazan colour disappeared. The yellow solution obtained after filtration was diluted with water, again filtered, acidified with concentrated hydrochloric acid and the methanol removed by distillation. The residual oil rapidly solidified and the product, 2:5-diphenyl - 3 - (4¹-styrylphenyl) - tetrazolium chloride, m.p. 228—229° C. (decomp.), recrystallised from water.

The intermediate formazan was prepared as follows:—4 - Aminostilbene (16.5 g.) in concentrated hydrochloric acid (40 c.c.) was treated with sodium nitrite (6.7 g.) dissolved in a little water, the temperature being kept below 5° C. The yellow diazonium suspension was added, with stirring, to benzaldehyde phenylhydrazone (16.4 g.) in pyridine (300 c.c.) at 0° C. After 2 hours, the black precipitate was collected, washed with water, alcohol and ether and recrystallised from ethyl acetate to give the deep red formazan, m.p. 225° C.

Similarly prepared, but in most cases precipitated as the iodides, were:—

1) 2:5 - Diphenyl -3- [4¹ - (4¹¹ - acetylamino - styryl) - phenyl] - tetrazolium iodide monohydrate, m.p. 244° C. (decomp.), from 1:3-diphenyl - 5 - [4¹-(4¹¹-acetylaminostyryl)-phenyl]-formazan, m.p. 208—209° C.

2) 2:5 - Diphenyl - 3 - [4¹ - (4¹¹-bromostyryl)-phenyl] - tetrazolium chloride monohydrate, m.p. 216—217° C. (decomp.), from 1:3 - diphenyl - 5 - [4¹ - (4¹¹ - bromostyryl)-phenyl]-formazan, m.p. 186—187° C.

3) 2:5 - Diphenyl - 5 - [4¹-(4¹¹-hydroxystyryl)-phenyl]-tetrazolium iodide, m.p. 272° C. (decomp.) from 1:3-diphenyl-5-[4¹-(4¹¹-bromostyryl)-phenyl]-formazan, m.p. 175—176° C.

4) 2 - Phenyl - 5 - (4¹ - bromophenyl)-3-(4¹ -styrylphenyl)-tetrazolium iodide, m.p. 206° C. (decomp.), from 1 - phenyl-3-(4¹-bromophenyl) - 5-(4¹- styrylphenyl)-formazan, m.p. 170—171° C. (decomp).

5) 2-Phenyl- 5 -(4¹-methoxyphenyl)-3-(4¹-styrylphenyl)-tetrazolium iodide, m.p. 167—168° C. (decomp.), from 1-phenyl- 3 -(4¹-methoxyphenyl)- 5 -(4¹-styrylphenyl) - formazan, m.p. 157—158° C.

6) 2:5-Diphenyl- 3 -[4¹-(4¹¹ - nitrostyryl)-phenyl] - tetrazolium chloride dihydrate, m.p. 233—234° C. (decomp.), from 1:3-diphenyl-5-[4¹-(4¹¹-nitrostyryl)-phenyl]-formazan, m.p. 185—186° C.

7) 2-Phenyl- 5 -methyl-3-(4¹-styrylphenyl)-tetrazolium iodide hemihydrate, m.p. 169—171° C., from 1-phenyl-3-methyl-5-(4¹-styrylphenyl) - formazan, m.p. 160—162° C. (decomp.).

8) 2-Phenyl- 5 -methyl- 3 -[4¹-(4¹¹-nitrostyryl)-phenyl]-tetrazolium iodide, m.p. 222—223° C. (decomp.), from 1-phenyl-3-methyl-5 - [4¹ - (4¹¹-nitrophenyl)-phenyl] - formazan, m.p. 182—183° C.

EXAMPLE VIII

1-(4¹-Phenylazophenyl)- 3 -phenyl- 5 -(4¹-styrylphenyl)-formazan (4 g.) was refluxed in methanol (60 c.c.) with yellow mercuric oxide (15 g.) after adding a little methanol, until the colour was discharged. After filtration, the solution was poured into dilute hydroiodic acid, giving a red tar which was crystallised from ethanol after charcoaling, yielding 2-(4¹-phenylazophenyl) - 5 - phenyl - 3 - (4¹-styrylphenyl)-tetrazolium iodide, m.p. 172—176° C. (decomp.).

The intermediate formazan was prepared as follows:—4 - Aminostilbene (5 g.) was diazotised with sodium nitrite (2 g.) and concentrated hydrochloric acid (10 c.c.) and coupled in the usual way with benzaldehyde phenylazophenylhydrazone (Tröger, Berlin and Franke, *Arch. Pharm., Bert. 244,* 326 (1906)) in pyridine (50 c.c.). The product was recrystallised from cyclohexane as purplish black needles.

EXAMPLE IX.

1-(4¹-Phenylazophenyl) - 3 - (4¹ - acetoxyphenyl)-5-(4¹-styrylphenyl)-formazan (2.0 g.) was reacted in ethanol (40 c.c.) with *iso*-amyl nitrite (4 c.c.) and hydrogen chloride gas until decolourised. The solution was poured into water, filtered and warmed with dilute hydrochloric acid. The residual gum was eluted with hot acetone and precipitated with ether. 2-(4¹-Phenylazophenyl) - 3 - (4¹-styrylphenyl)-5 - (4¹-hydroxyphenyl) - tetrazolium chloride was crystallised from acetone/ether containing a few drops of methanol m.p. 215—216° C.

The intermediate formazan was prepared as follows:—4¹-acetoxybenzaldehyde 4-phenylazophenylhydrazone was prepared by suspending 4-acetoxybenzaldehyde (22 g.) and finely powdered 4-phenylazophenylhydrazine β-sulphonic acid (36 g.) (Tröger and Franke, *Arch. Pharm., Berl., 244,* 307 (1906)) in glacial acetic acid (100 c.c.) and allowing to stand overnight, filtering the purple product, suspending in ice-water with excess sodium acetate, adding dilute ammonia solution until just alkaline, and filtering off. It was obtained as an orange solid, crystallising from benzene, m.p. 161—162° C. The formazan was prepared in the usual way by diazotising 4-aminostilbene (4 g.) in 50% concentrated hydrochloric acid (14 c.c.) and coupling with 4¹ - acetoxybenzaldehyde phenylazophenylhydrazone (6 g.) in pyridine (100 c.c.) as a dark purple solid, recrystallised from benzene, m.p. 220—221° C.

EXAMPLE X

1-(4¹-Phenylazophenyl) - 3 - (4¹ - carboxyphenyl)-5-(4¹-styrylphenyl)-formazan (18.5 g.) was oxidised in the usual way with *iso*-amyl nitrite and hydrogen chloride to yield 2-(4¹-phenylazophenyl) - 3 - (4¹-styrylphenyl)-5-(4¹-carboxyphenyl)-tetrazolium chloride as a red microcrystalline solid, recrystallised from ethanol, m.p. 195—196° C. (decomp.).

The intermediate formazan was prepared as follows:—Terephthalaldehydic acid 4-phenylazophenylhydrazone ammonium salt was prepared by a method similar to that used in the previous example from terephthalaldehydic acid (7.5 g.) and 4-phenylazophenylhydrazine β-sulphonic acid (14.8 g.) in warm glacial acetic acid (150 c.c.). It is a bright orange microcrystalline solid, m.p. 245° C. This ammonium salt (20 g.) was triturated with dilute hydrochloric acid and the product, terephthalaldehydic acid phenylazophenylhydrazone, was coupled in pyridine with diazotised 4-aminostilbene (13.3 g.), to yield 1 - (4¹ - phenylazophenyl) - 3 - (4¹ - carboxyphenyl)-5-(4¹-styrylphenyl) - formazan, dead-black small crystals, m.p. 239—240° C.

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